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Comparative toxicosis of sodium selenite and selenomethionine in lambs

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Abstract. Excess consumption of selenium (Se) accumulator plants can result in selenium intoxication. The objective of the study reported here was to compare the acute toxicosis caused by organic selenium (selenomethionine) found in plants with that caused by the supplemental, inorganic form of selenium (sodium selenite). Lambs were orally administered a single dose of selenium as either sodium selenite or selenomethionine and were monitored for 7 days, after which they were euthanized and necropsied. Twelve randomly assigned treatment groups consisted of animals given 0, 1, 2, 3, or 4 mg of Se/kg of body weight as sodium selenite, or 0, 1, 2, 3, 4, 6, or 8 mg of Se/kg as selenomethionine. Sodium selenite at dosages of 2, 3, and 4 mg/kg, as well as selenomethionine at dosages of 4, 6, and 8 mg/kg resulted in tachypnea and/or respiratory distress following minimal exercise. Severity and time to recovery varied, and were dose dependent. Major histopathologic findings in animals of the high-dose groups included multifocal myocardial necrosis and pulmonary alveolar vasculitis with pulmonary edema and hemorrhage. Analysis of liver, kidney cortex, heart, blood, and serum revealed linear, dose-dependent increases in selenium concentration. However, tissue selenium concentration in selenomethionine-treated lambs were significantly greater than that in lambs treated with equivalent doses of sodium selenite. To estimate the oxidative effects of these selenium compounds in vivo, liver vitamin E concentration also was measured. Sodium selenite, but not selenomethionine administration resulted in decreased liver vitamin E concentration. Results of this study indicate that the chemical form of the ingested Se must be known to adequately interpret tissue, blood, and serum Se concentrations.

Key words: Selenium, selenomethionine, sheep, sodium selenite toxicosis, vitamin E.

Introduction

Selenium (Se) is an essential trace element that plays a vital role in various physiologic processes. It is the only essential nutrient that does not have a generally regarded as safe status, and the FDA regulates Se supplementation in animals as if it were a drug.[13] In animals, there is a fairly narrow margin between Se deficiency and Se toxicosis. Diets containing Se concentration <0.1 part per million (ppm) can cause Se deficiency, whereas concentration >2.2 ppm may be toxic.[33] Inorganic Se such as sodium selenite (Na_2SeO_3) is commonly used, along with vitamin E for supplementation in animals diagnosed with Se deficiency, or in animals residing in Se-deficient areas.

Many human and animal cases of Se poisoning have been reported. Oral ingestion of 1 to 2.2 mg of Se/kg of body weight (BW) as sodium selenite has caused appreciable mortality in lambs up to 14 weeks of age.[14] In sheep, the oral median lethal dose (LD_{50}) of sodium selenite has been reported to be 1.9 ± 1.2 mg of Se/kg BW.[7,23] For intramuscularly administered sodium selenite, LD_{50}s have been reported to range from 0.45 to 1 mg of Se/kg BW.[3] Most losses from such acute exposure occur within 2 to 3 days. Acute selenosis results in depression, dyspnea, and death.[3] Chronic Se toxicosis, sometimes referred to as alkali disease, generally causes reduced weight, hair loss, and hoof abnormalities.[41]

Several hundred deaths have been reported in sheep from acute/subacute selenium intoxication following grazing of seleniferous plants growing on reclaimed phosphate mines in southeastern Idaho.[12] Although much of the published experimental work on selenosis in herbivores is based on studies of inorganic forms of Se, principally sodium selenite, the predominant chemical form of Se in plants is an organic form, selenomethionine (SeMet).[23,29,33] Considerable differences exist between the metabolic pathways of organic and inorganic Se compounds in the body.[15,18,19] The absorption of SeMet is greater, compared with that of sodium selenite.[38,39] Most of the Se from selenite forms physiologically functional selenoproteins, whereas a substantial portion of the Se from SeMet is also incorporated nonspecifically into nonfunctional or structural proteins.[17] Therefore, use of inorganic Se in the form of selenite as a standard compound for all Se investigations and risk assessments is not justified.[40]

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Table 1. Group-wise assignment of dosage and type of selenium (Se) in lambs.

Treatment	Se dosage	Amount and type of dosing compound
Group 1 (CTL*)	0 mg of Se/kg BW	Water only
Group 2	1 mg of Se/kg BW	2.19 mg of sodium selenite/kg BW
Group 3	2 mg of Se/kg BW	4.38 mg of sodium selenite/kg BW
Group 4	3 mg of Se/kg BW	6.57 mg of sodium selenite/kg BW
Group 5	4 mg of Se/kg BW	8.76 mg of sodium selenite/kg BW
Group 6 (CTL*)	0 mg of Se/kg BW	9.93 mg of methionine/kg BW
Group 7	1 mg of Se/kg BW	2.48 mg of selenomethionine/kg BW
Group 8	2 mg of Se/kg BW	4.97 mg of selenomethionine/kg BW
Group 9	3 mg of Se/kg BW	7.45 mg of selenomethionine/kg BW
Group 10	4 mg of Se/kg BW	9.93 mg of selenomethionine/kg BW
Group 11	6 mg of Se/kg BW	14.90 mg of selenomethionine/kg BW
Group 12	8 mg of Se/kg BW	19.86 mg of selenomethionine/kg BW

* CTL, control; BW, body weight.

The pharmacokinetics of toxic, intramuscularly administered doses of sodium selenite has been reported[4]; however, to the authors' knowledge, such studies have not been performed using intramuscularly or orally administered SeMet. Furthermore, comparison of tissue accumulation, pathologic changes, and oral dose kinetics is needed for the differentiation and better understanding of toxicoses caused by inorganic and organic Se. The purpose of the study reported here was to provide scientific data to address these issues. It was hypothesized that SeMet would be less toxic but more bioavailable than sodium selenite, and that SeMet would be retained in the tissues at a higher concentration. On the basis of results of previously reported in vitro experiments, it was also hypothesized that a decrease in vitamin E concentration in the sheep liver would occur after selenium oversupplementation.

Materials and methods

Animals. Forty-two crossbred lambs were acclimatized to the local conditions for 1 month, before administration of the Se compounds. During the entire acclimatization period, all animals had unlimited access to water, long-stem, alfalfa-grass hay, and trace mineral blocks.[a] All animals were 8 to 12 weeks old, weighed between 23 and 38 kg, and appeared healthy. The experiments reported here were reviewed and approved by the Institutional Animal Care and Use Committee, Utah State University, Logan, UT.

Experiment setup. After acclimatization, the 42 lambs were randomly assigned to 12 groups of 4 animals each, with 2 of either sex in each group, except for groups 5, 11, and 12, in which there were only 2 animals (Table 1). The lambs were weighed, and serum and blood samples were obtained from each animal 24 hours before Se dosing. Bacteriologic-grade sodium selenite[b] and seleno-DL-methionine[c] were used to prepare the dosing solutions, which were administered as a single intraruminal bolus. Each dose was prepared on the basis of BW of the animal, and was added to an empty 15-ml trace mineral-grade polypropylene tube. Just before administration of the dose, water was added to the tubes and the mixture was vortexed. This preparation was immediately delivered directly into the rumen through an intragastric tube. Finally, a water pump was connected to the intragastric tube, and water was flushed through it to ensure that no Se remained in the tube. This process was repeated for each lamb. At the end of the 7-day study, all lambs were weighed, then were euthanized by administration of a pentobarbital overdose followed by electrocution. Various internal organs were weighed. Heart, liver, and kidney were collected at necropsy and were stored at −70°C for analysis at a later time. Serum was also frozen at −70°C, whereas whole blood (heparinized) was stored at 4°C until analyzed. Complete physiologic sets of tissue samples were collected for histologic examination. The dosages were selected to induce substantial clinical effects, and to compare the findings with earlier reports of acute sodium selenite toxicosis.

Digestion of tissues for inductively coupled plasma mass spectrometry analysis. One gram (wet weight) of each tissue was put into a labeled 10-ml Oak Ridge Teflon digestion tube,[d] 2 ml of trace metal-grade nitric acid[e] was added, and the cap was attached. The tubes were heated at 90°C for 2 hours, with intermittent unscrewing of caps to release the pressure buildup and to avoid having them boil over. The tubes were then allowed to cool down, and volume of the contents was brought up to 3 ml by adding trace mineral-grade nitric acid. The contents were subsequently transferred to polypropylene, trace metal-free centrifuge tubes,[f] and 0.5 ml of this digest was pipetted into another trace metal-free tube containing 9.5 ml of ultrapure water obtained from use of an EASYpure UV/UF D8611[g] instrument. This resulted in a 5% nitric acid matrix, which provided a matrix match for the standard addition Se solutions.[h] After vortexing, the samples were analyzed using an inductively coupled plasma mass spectrometer (ICP-MS).[i]

Digestion of blood/serum for ICP-MS analysis. Seven-hundred fifty microliters of the sample was introduced into a labeled 10-ml Oak Ridge Teflon digestion tube.[d] An equal amount (750 μl) of trace mineral-grade nitric acid was added to the digestion tubes, and the caps were sealed. The tubes were then heated at 90°C for 2 hours without unscrewing of the caps. After digestion, tubes were allowed to cool down, and contents were transferred to another trace metal-free tube. One milliliter of this digest (750 μl of sample + 750

μl of nitric acid) was transferred into another trace metal-free tube containing 9.0 ml of ultrapure water, to make up a 5% nitric acid matrix. After vortexing, the samples were analyzed by use of ICP-MS.

Procedure for selenium analysis. Samples prepared as per the aforementioned digestion methods were analyzed using the ELAN 6000 ICP-MS.[i] Quantification of Se was performed by the standard addition method, using a 4-point standard curve. A quality-control sample (in similar matrix) was analyzed after every 5 samples, and analysis was considered acceptable if the Se concentration of the quality-control sample fell within ±5% of the standard/reference value for the quality control.

Vitamin E quantification in liver. A high-performance liquid chromatographic (HPLC)[j] system with 100-μl sample loop, and a model 046-A programmable fluorescent detector (FLD) was used, and fitted with an HP ODS Hypersil 5-μm-thick, 125 × 4-mm-long column. The mobile phase was 3% methanol in water, and the flow rate was kept at 1 ml/min. The column temperature was constant at 35°C. The FLD excitation and emission wavelengths were 295 and 325 nm, respectively. The following reagents were required for vitamin E analysis: ascorbic acid in absolute ethanol (2% solution), saturated potassium chloride solution (aqueous), petroleum ether[c] (35 to 60°C), HPLC-grade methanol[c], and vitamin E[k] standard (diluted to 10 ppm in methanol and kept refrigerated in an amber bottle).

Procedure for vitamin E analysis. Two grams of minced liver sample (in duplicate) was placed into 50-ml centrifuge tubes with Teflon screw caps. One of the duplicates was spiked with 2.5 ppm of vitamin E standard (250 μl). Then, 5 ml of 2% ascorbic acid in ethanol was added to the tubes, followed by 1 ml of saturated potassium chloride solution. This mixture was homogenized in an ice bath for 4 minutes by use of a biohomogenizer. After homogenization, 10 ml of petroleum ether was added to the tubes, which were then vortexed for 2 to 3 minutes. This material was centrifuged at 1,000 ×*g* for 5 minutes, and the petroleum ether layer was transferred to a 10-ml amber tube. The ether was evaporated under nitrogen gas, with no heat. The petroleum ether extraction step was repeated. Residue after the 2 extractions was reconstituted in 1 ml of methanol and was centrifuged at 500 ×*g* for 10 minutes. Finally, the supernatant was transferred to a 2-ml, capped Eppendorf tube, and was analyzed.

Analysis of vitamin E in liver. Two hundred-microliter samples of each vitamin E standard in methanol (0, 1.25, 2.5, and 5 ppm) were injected into the HPLC to obtain standard curves. This was followed by injection of a blank, then 0.2 ml of each of the prepared samples. The retention time or the vitamin E peak was obtained at 4.5 minutes. Recovery (%) was calculated using the following formula:

$$[(\text{Area of Vit E peak from spiked sample} - \text{Area of Vit E peak from unspiked sample}) \div (\text{Area of the 2.5 ppm Vit E standard})] \times 100$$

Statistical analysis. The collected data were processed by use of analysis of variance (ANOVA) with a general linear model (GLM) procedure in SAS.[l] Multiple comparisons of main effects as well as the interactions were performed using the Least Square Means (LSMEANS) statement. Significance was set at $P < 0.05$.

Results

Lambs belonging to the groups given Se at dosages of 2, 3, and 4 mg/kg BW as sodium selenite and 4, 6, and 8 mg Se/kg BW as SeMet had visible evidence of reduced feed intake, depression, reluctance to move, and tachypnea following minimal exercise. These animals stood with their head down and neck extended, taking short, rapid, shallow breaths. The onset of clinical signs of disease was observed 12 to 24 hours after Se dosing, and the recovery period ranged from 2 to 3 days. Severity of clinical signs of disease and time to recovery varied and were dose dependent. At necropsy 7 days after dosing, 1 animal each from the 2 and 3 mg of Se/kg BW selenite groups, and all animals from the 4 mg of Se/kg BW selenite group and from the 6 and 8 mg of Se/kg BW SeMet groups had severe pulmonary lesions characterized by marked interlobular edema (Fig. 1) and accumulations of serosanguinous fluid and foam in the trachea, bronchi, and bronchioles. The heart of these animals was soft, pale, and mottled red. The liver was red and mildly swollen. Groups given 0 and 1 mg of Se/kg BW as sodium selenite and 0 to 3 mg of Se as SeMet did not have remarkable clinical signs of disease or gross lesions. Two lambs (1 each from the 6 and 8 mg of Se/kg BW SeMet groups) became severely ill and were euthanized at 24 and 36 hours after dosing, respectively, followed by necropsy. These lambs were dull, ataxic, and had substantial wheezing and respiratory grunts (dyspnea) for an hour; this was immediately followed by recumbency and euthanasia.

The major histopathologic changes in lambs that manifested clinical signs of disease included acute multifocal myocardial necrosis and pulmonary alveolar vasculitis with pulmonary edema and hemorrhage. The myocardial lesions involved multifocal, randomly distributed zones of acute monophasic necrosis (Fig. 2). The necrosis was characterized by myofiber swelling, with coagulation of sarcoplasmic proteins. Some areas had focal accumulations of macrophages and lymphocytes. There was no evidence of mineralization. The pulmonary lesions were characterized as multifocal perivascular edema, with minimal accumulations of neutrophils and fibrin within some alveolar septae. Some areas had small hemorrhages that occasionally filled the alveoli. The liver was mildly congested. Affected lambs of the higher dosage groups (2, 3, and 4 mg/kg BW as sodium selenite, and 4, 6, and 8 mg/kg BW as SeMet) had mild multifocal interstitial pneumonia characterized by focal vasculitis, with mild interstitial edema and congestion (Fig. 3). Many of these lambs also had mild multifocal swelling

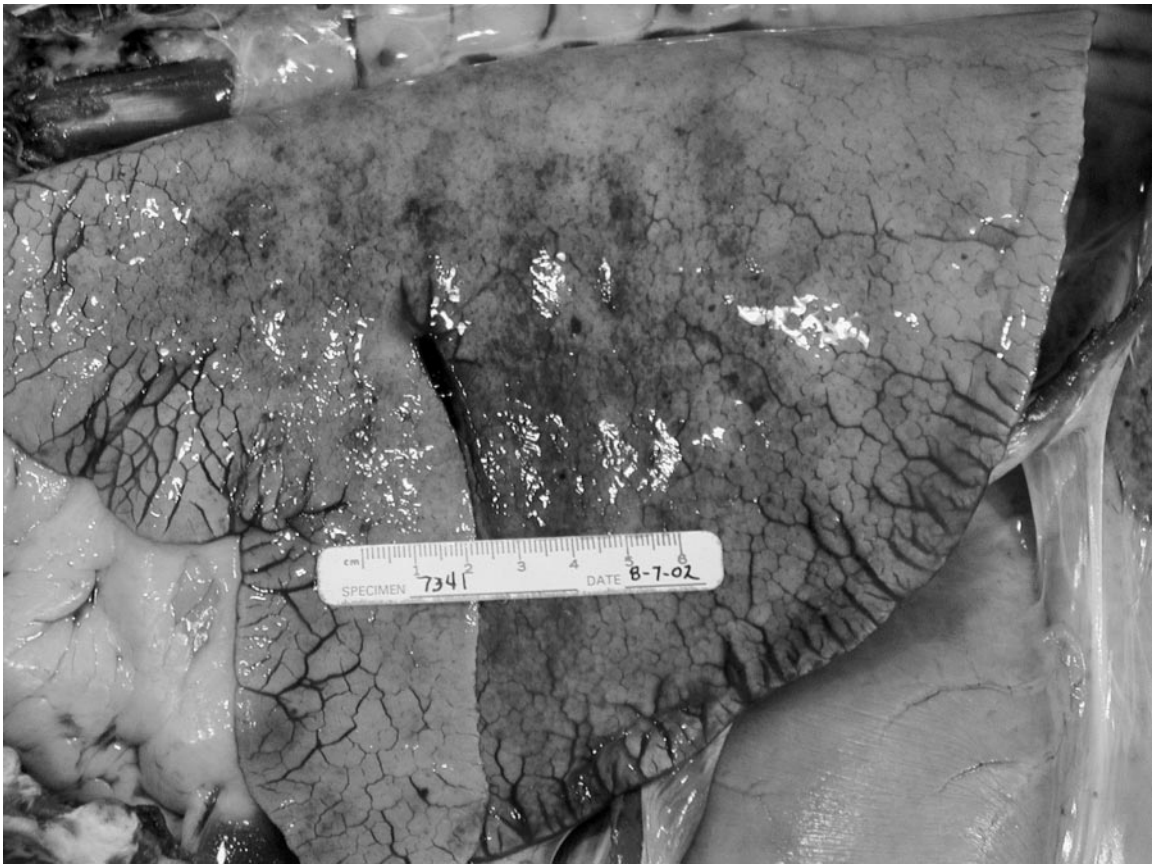

Figure 1. Lung from a lamb that was treated with 6 mg of selenium (Se)/kg of body weight (BW) as selenomethionine. Notice severe interlobular and subcapsular edema. Bar = 1 cm.

and separation of myocardial myofibers. Some areas had small accumulations of lymphocytes in the adjacent myocardial interstitium. Relevant histologic lesions were not found in multiple sections of brain, liver, kidney, adrenal gland, lymph node, spleen, pancreas, thyroid, skeletal muscle, rumen, abomasum, duodenum, jejunum, ileum, colon, or cecum. All control animals were clinically and histologically normal.

At 7 days after dosing, a significant effect was not observed on total body weight, or weight of internal organs such as the lungs, heart, liver, spleen, kidneys, thyroid, right mandibular lymph gland, or adrenal glands. The Se concentrations in liver, kidney cortex, and heart muscle of the various groups are presented in Table 2. Liver Se concentration in groups given 0 to 4 mg of Se/kg BW as sodium selenite was significantly different from that in groups given equivalent Se doses as SeMet ($P < 0.0001$). Liver Se concentrations in lambs of each sodium selenite group also were significantly different from each other, with the exception that, in the lambs of the 1 mg of Se/kg BW sodium selenite group, liver Se concentration was higher than that in lambs of the control group, but the difference was not statistically significant. For SeMet, liver Se values for all Se-treated and control groups were statistically different from each other. The kidney cortex Se concentration in groups given 0 to 4 mg of Se/kg BW as sodium selenite also was significantly different from values for the SeMet groups ($P < 0.0018$). Values for the 3 and 4 mg of Se/kg BW sodium selenite groups were significantly different from values for controls and from values for each other. For SeMet, values for all groups were significantly different from values for each other.

The atrial and ventricular muscles of the heart were separately analyzed for Se content. In groups given Se as sodium selenite ($P = 0.01$) and SeMet ($P = 0.0002$), the Se concentration in the ventricles was significantly higher than the concentration in the atrium. A linear, dose-dependent, Se accumulation pattern was observed in all of the tissues evaluated for both Se forms.

The Se concentration in serum increased linearly with dosage (Fig. 4). Serum dose-response relationships for sodium selenite and SeMet were significantly different ($P < 0.0001$). Groups given 3 and 4 mg Se/kg BW as sodium selenite had significantly higher serum Se concentration than that in the controls. The Se

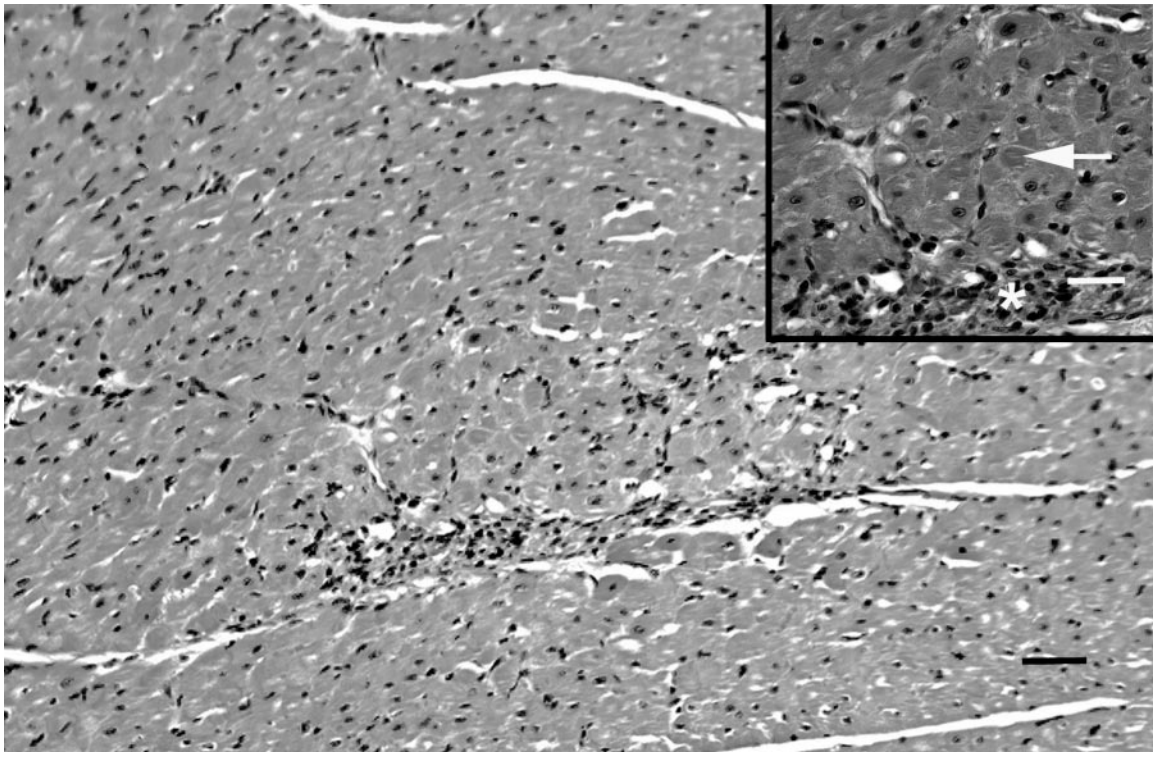

Figure 2. Photomicrograph of a section of the heart from a lamb that was treated with 6 mg of Se/kg BW as selenomethionine. Notice acute myocardial necrosis with minimal lymphocytic inflammation (*inset). Arrows (inset) are coagulated sarcoplasmic proteins. HE. Bar = 150 μm and bar (inset) = 100 μm.

concentrations in serum for all SeMet treatments were significantly different from each other, and from values for the controls. The Se concentration in blood followed a similar trend, with groups given 3 and 4 mg of Se/kg BW as sodium selenite having a significantly higher blood Se concentration than that of controls ($P = 0.0133$). Whole-blood dose-response relationships for selenite and SeMet treatments were significantly different. The Se concentrations for all SeMet treatments were significantly different from each other, with the exception of the group given 1 mg of Se/kg BW as SeMet, the value for which was higher, but not statistically different from the value for controls (Fig. 5). From the serum and whole-blood values, the Se concentration within the erythrocytes was calculated using the following formula: Erythrocyte Se (ppm) = ([whole-blood Se {ppm}] − [0.6 × serum Se {ppm}]) × 2.5, assuming PCV of 40%,[5] and the remainder of the whole blood as serum. Increasing the dosage of sodium selenite did not have any effect on Se concentration in the erythrocytes ($P = 0.502$), and the Se values were statistically similar to those for the controls. However, the erythrocyte Se concentration in the SeMet-treated groups increased linearly, in dose-dependent manner (Fig. 6. Values for groups given 2, 3, and 4 mg of Se/kg BW as SeMet were significantly higher than values for the controls ($P < 0.0001$).

Analysis of vitamin E concentrations in the liver of lambs treated with various dosages of sodium selenite is presented in Table 3. Recovery of the added Se (spiked) ranged from 85 to 110%. One data point belonging to the 3 mg/kg BW selenite group was statistically identified as a true outlier and was removed from the analysis. The overall R^2 was 62%, and results of the ANOVA analysis suggested a significant effect ($P = 0.0151$) at the 95% confidence level. Similar analysis of the SeMet-treated groups indicated no significant effect ($P = 0.6776$). The group given 8 mg of Se/kg BW as SeMet had lower vitamin E concentration in the liver, but the difference was not statistically significant. The controls for the sodium selenite and SeMet treatments had statistically similar concentrations of vitamin E in the liver.

Discussion

On the basis of earlier reported toxic dosages that ranged from 1 to 2.2 mg/kg Se BW as sodium selenite,[7,14,23] we expected to see lethality in the high-dose sodium selenite groups. Clinical effects were observed, but without lethality. Unexplained variations of Se toxicosis have been reported in sheep.[14,34] Some sheep are inherently susceptible to Se toxicosis,[25] and stress factors such as exercise can render an animal more susceptible.[14] In the rumen, variable fractions of

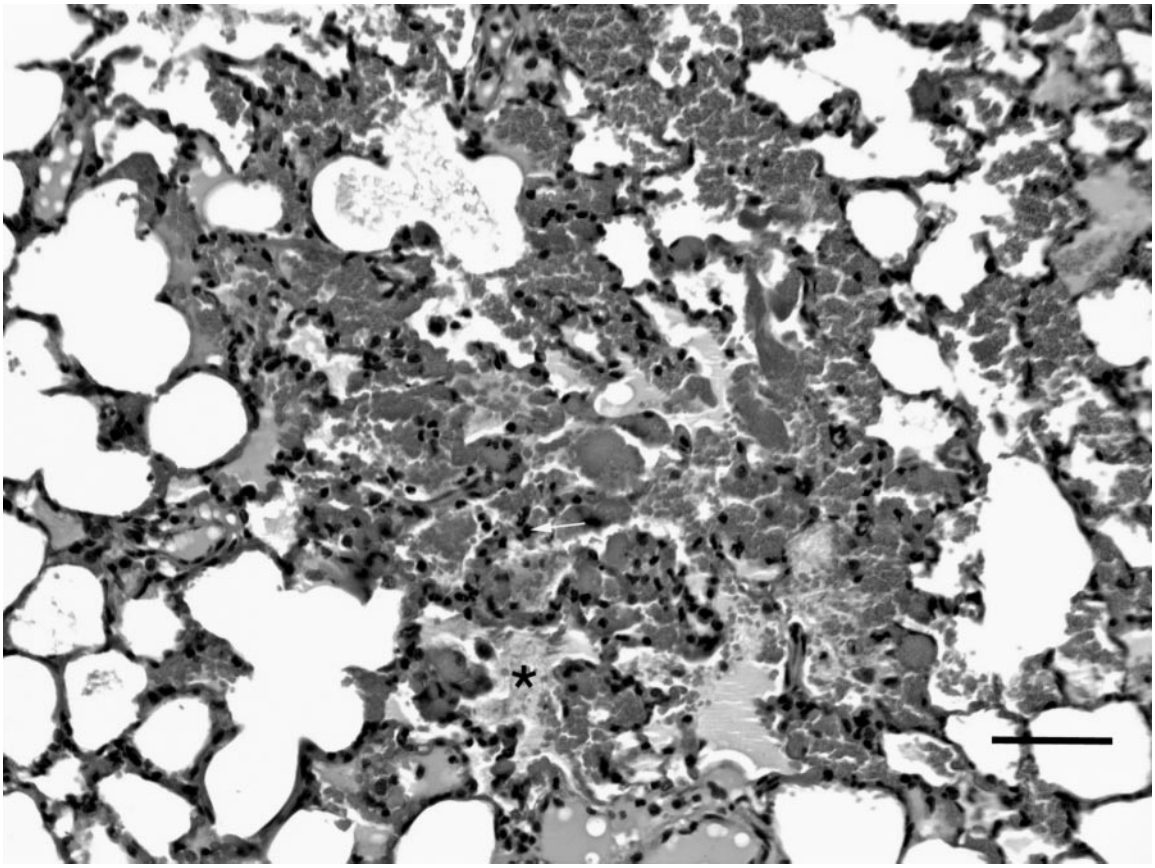

Figure 3. Photomicrograph of a section of the lung from a lamb that was treated with 6 mg of Se/kg BW selenium as selenomethionine. Notice severe pulmonary congestion, minimal vasculitis, and interstitial and alveolar hemorrhages. HE. Bar = 100 μm.

Table 2. Liver, kidney, and heart Se concentrations (ppm wet weight) at 7 days after dosing in lambs administered different forms and amounts of selenium (Se).*

Se dosage†	Liver (mean ± SD)	Kidney cortex (mean ± SD)	Heart (atrium and ventricles) (mean ± SD)	
Se administered as sodium selenite				
0 mg (group 1)	0.237 ± 0.037	1.223 ± 0.081	0.209 ± 0.022	0.210 ± 0.014
1 mg (group 2)	0.955 ± 0.108	1.593 ± 0.145	0.270 ± 0.018	0.300 ± 0.006
2 mg (group 3)	2.088 ± 0.766	1.661 ± 0.347	0.345 ± 0.008	0.370 ± 0.061
3 mg (group 4)	3.978 ± 0.495	2.203 ± 0.324	0.389 ± 0.013	0.433 ± 0.041
4 mg (group 5)	6.726 ± 0.171	2.487 ± 0.724	0.431 ± 0.027	0.518 ± 0.066
Se administered as selenomethionine				
0 mg (group 6)	0.213 ± 0.015	1.135 ± 0.059	0.200 ± 0.028	0.207 ± 0.023
1 mg (group 7	2.196 ± 0.813	1.819 ± 0.178	0.464 ± 0.062	0.576 ± 0.043
2 mg (group 8)	5.070 ± 0.751	2.370 ± 0.227	0.775 ± 0.184	0.926 ± 0.191
3 mg (group 9)	8.871 ± 0.813	3.011 ± 0.318	1.029 ± 0.041	1.296 ± 0.101
4 mg (group 10)	10.800 ± 0.764	4.019 ± 0.132	1.351 ± 0.182	1.726 ± 0.120
6 mg (group 11)	14.780 (25.99‡)	4.501 (11.187‡)	1.592 (3.851‡)	1.968 (4.125‡)
8 mg (group 12)	15.573 (23.42§)	5.248 (12.873§)	1.986 (2.905§)	2.576 (4.346§)

* n = 4 for each group (except groups 5, 11 and 12, which had 2 lambs).
† Mg of Se/kg BW.
‡ Se concentration in the tissue from a lamb euthanized at 24 hours after dosing.
§ Se concentration in the tissue from a lamb euthanized at 14 hours after dosing.

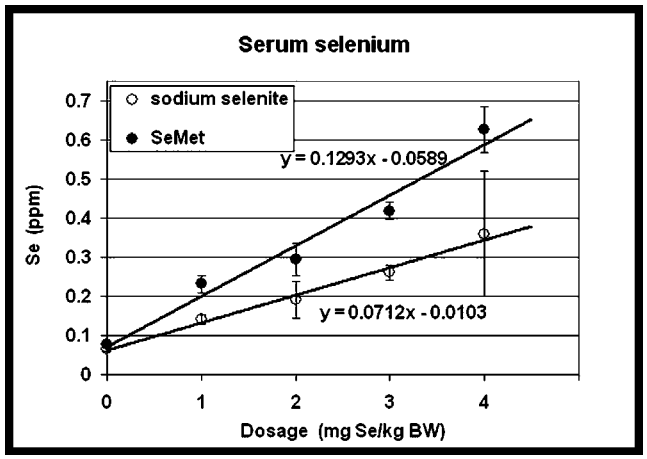

Figure 4. Selenium (Se) concentrations in serum of lambs 7 days after being administered 0 to 4 mg of Se/kg BW as sodium selenite or selenomethionine.

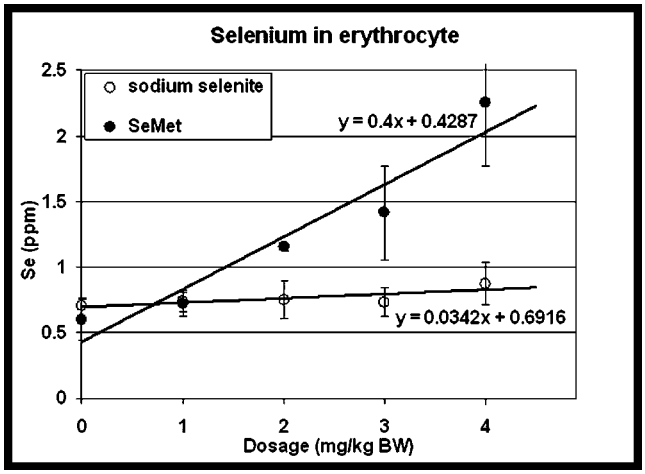

Figure 6. Selenium (Se) concentrations (wet weight basis) in red blood cells (RBCs) of lambs 7 days after being administered 0 to 4 mg of Se/kg BW as sodium selenite or selenomethionine.

selenate and selenite are reduced to insoluble selenides and elemental Se that are considered a nonbioavailable Se sink. Because of the predominance of certain bacteria, such as *Bacteroides rumenicola*, that reduce Se compounds to the elemental form, decreased Se absorption has been observed in sheep fed a forage-based diet.[34] Other microbes such as *Selenomonas ruminantium, Wolinella succinogenes,* and *Prevolella ruminicola* are examples of other ruminal bacteria that use selenite, and together with hydrogen sulfide and cysteine, convert the bioavailable selenite to nonbioavailable elemental Se or Se sulfide. Compared with those of earlier reports, the lambs of this study appeared to have relatively higher resistance toward Se toxicosis.

In general, the absorption and retention of Se in the form of amino acids such as SeMet and selenocysteine is greater, compared with the absorption and retention of inorganic species such as selenites.[38,39] This is reflected in the significantly higher mean Se concentration in tissues from the SeMet-treated groups, compared with the sodium selenite-treated groups. Most of the Se from ingested sodium selenite is present as selenoprotein-P (Sel-P) and glutathione peroxidase (GSH-Px).[2,26] On the other hand, ingested SeMet that is not immediately metabolized is incorporated into organs with a high rate of protein synthesis, such as skeletal muscle, forming up to 50% of the Se pool.[17] In the study reported here, higher Se concentration in the ventricles of the heart, versus the atrium, can be attributed to a higher protein turnover rate in the ventricles, which results in a greater amount of SeMet being deposited. Other tissues that tend to accumulate Se include erythrocytes, pancreas, liver, kidney, stomach, and the gastrointestinal tract mucosa.[16] Selenium is found in all tissues in concentrations that vary with the amount ingested in the diet and the type of tissue.

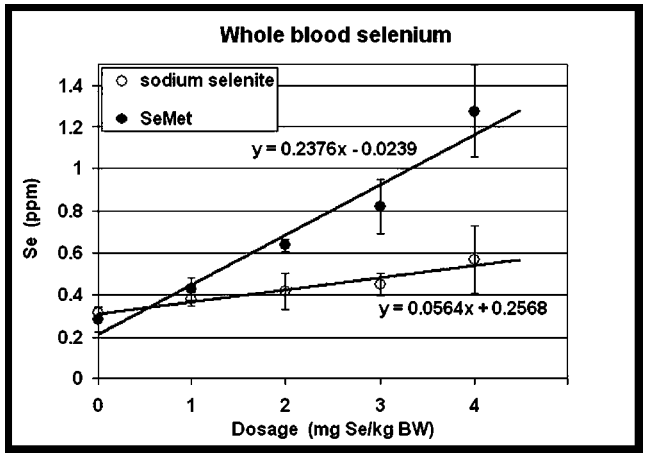

Figure 5. Selenium (Se) concentrations (wet weight basis) in blood of lambs 7 days after being administered 0 to 4 mg of Se/kg body weight as sodium selenite or selenomethionine.

Table 3. Effect of Se on vitamin (vit) E concentrations in the liver (wet wt basis).

Chemical form	Se dosage	Vit E (ppm in liver)	
Sodium selenite	− (control)	2.1 (± 0.3)	$n = 4$
Sodium selenite	1 mg/kg BW	1.9 (± 0.3)	$n = 4$
Sodium selenite	2 mg/kg BW	1.9 (± 0.2)	$n = 4$
Sodium selenite	3 mg/kg BW	1.4 (± 0.3)*	$n = 3$
Sodium selenite	4 mg/kg BW	1.4 (± 0.0)*	$n = 2$
Selenomethionine	(control)	2.0 (± 0.3)	$n = 4$
Selenomethionine	1 mg/kg BW	2.0 (± 0.3)	$n = 4$
Selenomethionine	2 mg/kg BW	1.9 (± 0.1)	$n = 4$
Selenomethionine	3 mg/kg BW	1.9 (± 0.3)	$n = 4$
Selenomethionine	4 mg/kg BW	1.9 (± 0.2)	$n = 4$
Selenomethionine	6 mg/kg BW	1.9 (± 0.1)	$n = 2$
Selenomethionine	8 mg/kg BW	1.6 (± 0.3)	$n = 2$

* Significantly lower than the value for sodium selenite controls ($P = 0.0151$; $\alpha = 0.05$).

Sheep not supplemented with Se can have higher concentration of it in the kidneys than in the liver, but with Se supplementation, the liver concentration increases disproportionately.[10] In one report, 3 sheep that were accidentally poisoned by sodium selenite had liver, kidney, and heart Se concentrations in ranges of 7.1 to 10.8, 3.3 to 5.5, and 3 to 5 ppm, respectively.[22] Normal reference intervals are 0.25 to 1.5 ppm in the liver, and 0.9 to 3 ppm in the kidney cortex of sheep.[12,31]

The 2 lambs that were euthanized early, 1 each from the 6 and 8 mg of Se/kg BW as SeMet groups, became severely ill within 24 hours of exposure. The observed clinical changes and lesions were consistent with those reported in association with acute sodium selenite toxicosis.[1,3,23,34] The gross and histologic lesions of the lambs of this study as well as those previously reported suggest that the heart is the target organ of Se intoxication.[34] The toxic damage to the myocardium associated with Se poisoning has been positively correlated with the Se concentration in the heart muscle itself.[18] The pulmonary edema and hydrothorax in these lambs could have been cardiogenic. Other investigators have suggested that acute selenium fatalities are attributable to cardiovascular compromise and respiratory failure, but the exact mechanism of action resulting in this pathologic scenario remains unknown.[32] The vascular changes observed in the lambs of this study suggest that the pulmonary edema was more than cardiogenic. Results of earlier experiments indicated that sodium selenite is a pro-oxidant compound in vivo.[11,35] We found that the vitamin E concentration in the liver of severely affected lambs given sodium selenite was significantly reduced, verifying those previous reports. We therefore speculate that Se-induced oxidation can significantly contribute to the pathogenesis of Se intoxication, at least for the inorganic form of selenium.

Significant increase in Se concentration was not observed in the red blood cells (RBCs) at 7 days after dosing of animals administered 0 to 4 mg of Se/kg BW as sodium selenite (Table 3). This is consistent with an earlier report indicating that selenite is rapidly taken up by the RBCs, but then is released after reduction to selenide, provided that enough reduced glutathione is available for this process.[37] On the other hand, SeMet can be incorporated directly into hemoglobin in the RBCs. Selenium is concentrated in erythrocytes, relative to the amount in blood plasma.[6] Selenium is incorporated into new maturing RBCs and remains there for the lifetime of the cell. In the bone marrow, Se is incorporated into the RBCs during erythropoiesis, predominantly as GSH-Px. Most GSH-Px activity in whole blood is contained within the RBCs, with a small fraction being free in plasma. Approximately 75 to 85% of the Se in ovine erythrocytes is associated with GSH-Px.[28] Previous reports indicated that complete blood count and serum biochemical values were not significantly affected after Se exposure.[12]

Vitamin E and Se complement each other in their function as antioxidants. Vitamin E prevents free radical production, whereas Se, as a component of glutathione peroxidase, neutralizes the free radicals that are present in the biological matrix.[9,11] Thus, vitamin E and Se have a sparing effect on each other. However, at high doses, selenite is reported to act as a pro-oxidant compound in vitro.[8,21,24,35,36] This property has not been identified for SeMet.[20,36] The cytotoxic effects of selenite in vitro, even though they are nonspecific, are protected by the simultaneous presence of vitamin E.[27] Supranutritional doses of vitamin E can reduce lipid peroxidation and mortality in selenite-treated animals.[32] Selenite has also been reported to stimulate lipid peroxidation in vivo.[11] By measuring the vitamin E concentration in the liver, we documented for the first time that vitamin E can be depleted by oversupplementation of Se as sodium selenite in vivo. From this analysis, it can be inferred that sodium selenite at higher dosages might have caused depletion of the vitamin E in the liver, an effect not observed in animals treated with equivalent amounts of Se in the form of SeMet. This can also contribute to the differences in the toxicity between sodium selenite and SeMet.

On the basis of the results from this study, it is concluded that, in an acute oral exposure, Se from SeMet can be twice as bioavailable, but can be slightly less toxic than sodium selenite. This can, in part, be attributable to the ingested Se from SeMet being directly incorporated into nonfunctional structural proteins as a direct methionine replacement.[30] However, judging the Se status of an animal by use of tissue concentrations could be problematic. Selenium-deficient animals administered SeMet can develop normal tissue Se concentrations, but a significant amount can be present in a structural/nonphysiologically active form such as a methionine replacement. This can compromise the primary role of Se as an antioxidant. Most of the reference intervals in feed and tissues have been based on results of studies involving sodium selenite, which follows an appreciably different metabolism and kinetics. The evaluation of animals fed a natural Se or SeMet supplemented diet can, at times, cause one to overlook a potential, underlying deficiency. On the other hand, SeMet has a higher potential of bioaccumulation and can pose a higher risk after multiple exposure to high-Se diets. The bioaccumulation of Se given as SeMet can be used advantageously in animals that require a sustained Se source, as Se is released from the structural selenoproteins in due course of protein turnover.

Blood remains the sample of choice for antemortem diagnosis of Se deficiency or chronic Se poisoning. However, in cases of acute Se poisoning, antemortem serum Se concentration appears to be a more sensitive indicator of overexposure to sodium selenite. In cases of acute SeMet intoxication, either blood or serum can be used as a diagnostic specimen. Hemolysis can increase serum Se concentration. Adequate Se concentration for sheep blood ranges from 0.08 to 0.5 ppm in whole blood and from 0.07 to 0.4 ppm in serum.[32] On the basis of results of this study, in cases of acute Se intoxication, comparative analysis of serum and whole-blood Se concentrations can help differentiate between inorganic sodium selenite and organic SeMet exposures. The low Se content of RBCs associated with high selenite intake versus the high Se RBC content associated with high SeMet intake provides a definitive contrast that can allow differentiation of the form of Se causing a poisoning incident. This is key information because a potentially toxic serum Se concentration following selenite ingestion can be lower than that resulting from SeMet ingestion.

Liver is considered the postmortem sample of choice for diagnosing Se deficiency or toxicosis. In addition to the route and dosage, the form of the Se exposure should also be considered when interpreting liver Se concentration. Without knowing the form of Se to which an animal might be exposed, a high Se value can be misinterpreted as being toxic.

From results of this study, we document the importance of knowing the administered form of Se when interpreting serum, whole-blood, and tissue data from cases of Se intoxication. Administration of SeMet results in higher tissue, serum, and whole-blood Se concentrations than does administration of equivalent doses of selenite. We have also documented that a diagnostician can presumptively differentiate between selenite and SeMet by use of comparative whole-blood versus RBC Se content. Further work will be performed to compare the effects of plant Se to those of purified selenite and SeMet.

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Sources and manufacturers

a. American Stockman, Overland Park, KS.
b. United States Biochemical Corporation, Cleveland, OH.
c. Sigma Chemical Co, St Louis, MO.
d. Nalge Nunc International, Rochester, NY.
e. Fisher Scientific, Pittsburgh, PA.
f. ELKAY, Mansfield, MA.
g. Barnstead | Thermolyne, Dubuque, IA.
h. Spex CertiPrep Inc, Metuchen, NJ.
i. Perkin Elmer, Shelton, CT.
j. Agilent Technologies, Palo Alto, CA.
k. General Biochemicals Inc, Chagrin Falls, OH.
l. SAS Institute, Cary, NC.

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